Diagnosis Procedure: Mycobacteriology

Diagnostic Procedure: Mycobacteriology
Specimen Collection

Collection of clinical specimens to obtain smears and cultures for *M. tuberculosis* is necessary and appropriate. Such specimens usually are sputum and other specimens from the respiratory tract, but in certain situations may include urine, cerebral spinal fluid, pleural fluid, purulent material from a needle aspiration or abscess, or biopsy specimens. **Tissue specimens must be placed in a sterile saline solution, NOT FORMALIN, and either examined immediately in a hospital laboratory or shipped by overnight express to the Missouri State Tuberculosis Laboratory.**

Diagnostic Procedure: Mycobacteriology
Sputum Collection

POLICY: Sputum specimens will be obtained in the following situations: Any person presenting themselves with an unexplained chronic (≥ 3 weeks) productive cough. Any person with newly diagnosed or suspected pulmonary tuberculosis. Specimens should be collected on **three (3) separate days each month until both smear and culture have converted to negative. A final series of three (3) sputum specimens collected on three separate days of the last month of treatment** provides laboratory evidence that the treatment regimen has been successful.

PURPOSE: Sputum examinations are an important tool in the establishment of the diagnosis of pulmonary disease caused by *Mycobacterium tuberculosis (M. Tb).* Response to treatment is monitored by the results of the sputum smears and cultures, which can also be used to help in determining the likelihood of infectiousness. Effectiveness of anti-tuberculosis therapy is also monitored by the sputum examinations.

PROCEDURE: **Spontaneously Produced Sputum:** Accuracy of the laboratory reports is directly dependent upon the quality of the specimen submitted. Instruct the patient and/or family regarding the importance of following the procedure exactly. Impress upon the patient that the specimen to be obtained is not saliva, but material from the lower air passages.

For specimens being submitted to the State Tuberculosis Laboratory at the Missouri Rehabilitation Center in Mount Vernon, sputum specimen containers and blank enclosure forms may be obtained from the LPHA. Clearly print all information needed on the enclosure form and on the outside of the mailing tube. Also the sputum container should be clearly labled with the patients name. For submission to commercial laboratories, specimen containers will be provided by those labs.

If the patient is unable to carry out the collection of sputum on his/her own, the person helping him/her should wear a N95 mask; properly fit tested according to Centers for Disease Control and Prevention (CDC) recommendations.

The patient should brush teeth, rinse mouth and throat with water, and wash hands before going to bed at night and again when first rising in the morning. He/she should then cough up the sputum from deep in the lung, collecting it in the sputum container. A volume of 5 to 10 ML is adequate for each sample. Keep the outside of the sputum bottle from becoming contaminated with sputum. Wipe the lip of the bottle with disposable tissue and cap the sputum container

tightly. Place in the metal screw-top container, with the completed laboratory slip placed between the metal container and the outside mailing tube. Stamp and mail immediately to the laboratory--DO NOT WAIT UNTIL ALL THREE SPECIMENS HAVE BEEN COLLECTED.

For specimens being sent to the State Tuberculosis Lab, Federal Express packaging and shipping is available at no charge to the LPHAs. The specimen kits will contain the following (IATA/ICAO approved) shipping containers, specimen containers, patient information sheet and return shipping supplies. The specimen kits can be obtained by the LPHAs by calling the State Tuberculosis Laboratory at 417-461-5391.

All isolates of *M. tuberculosis* will be called to the submitting health care provider as soon as the identification is made.

It is important to remember that the smear result will be available within 24 hours of the laboratory receiving the specimen; negative cultures will be reported in six (6) weeks.

The laboratory at Mount Vernon will routinely perform sensitivity studies to all five first-line anti-tuberculosis drugs on the first specimen that is culture-positive for *M. Tb.*

Sputums should be obtained one to three times per week if possible and until patient has converted to smear and culture negative. **24-hour sputum specimen collection is NOT an acceptable alternative.**

Techniques to Aid in the Collection of Sputum: It is sometimes difficult for patients to raise sputum. The following techniques may help.

1. Moist air inhalation, such as with a tea kettle (being careful to avoid burns), or a shower, may help to liquefy the sputum, increasing the volume and making it easier to expectorate.

2. Holding one nostril closed, inhale deeply through the open nostril and exhale through the mouth. Repeat twice, and on the third exhalation, give a deep cough. If necessary, repeat, until sputum is produced.

Sputum Induction: Sputum may be induced by use of **nebulized saline solution**. This requires special equipment available in some LPHAs, hospitals, and offices of health care providers. The resulting sputum specimen is often watery. THE SPECIMEN SHOULD BE LABELED "INDUCED."

When sputum is induced by use of a nebulizer, the cough produced may be violent in nature, and is often difficult to control and cover. Therefore, all such procedures must be performed in areas that have local exhaust ventilation devices (e.g., booths or special

enclosures) or in a room that meets the ventilation requirements for TB isolation. Persons who have had this procedure performed must remain in the booth or enclosure until their coughing subsides. Personnel should not enter the room without an approved mask until there has been sufficient time for the ventilation system to have removed at least 99 percent of the potentially infectious droplet nuclei, which will depend on the efficiency of the system. (See Chapter 8 of *Core Curriculum.*)

Tracheal Suction: Suctioning may be used for patients too ill to understand and/or cooperate with the collection of sputum. Suctioning may produce violent coughing, similar to the coughing produced by the **induction** method above, and personal protective masks (N95) (see Section 3.1) as recommended by Centers for Disease Control and Prevention and approved by the National Institute for Occupational Safety and Health must be worn by health care workers during the procedure. (See Chapter 8 of *Core Curriculum)*

Gastric aspiration may be necessary to obtain swallowed sputum specimens. It is a method used to obtain sputum from infants and small children unable to expectorate. Fasting, early morning specimens are recommended to obtain sputum swallowed while sleeping. Samples, which must be adjusted to neutral pH, should be collected on three consecutive days. If the specimen cannot be processed by a laboratory within 4 hours, the specimen must be collected in a sterile container with 100 mg of sodium carbonate.

No neutralized specimens are not accepted by the State Tuberculosis Laboratory. Neutralized specimens should be transferred to sputum containers and sent for testing. THE SPECIMEN SHOULD BE LABELED “GASTRIC”.

Diagnostic Procedure: Mycobacteriology
Bronchoscopy

Bronchoscopy may be utilized to obtain specimens of tissue and/or sputum for examination for acid-fast bacilli (AFB) and culture for the presence of mycobacteria. This procedure usually produces coughing and sputum production for hours, and personal protection masks (N95) must be worn by health care workers during the procedure.

Bronchoscopy should be performed in a room that meets AFB isolation requirements (see Chapter 8 of *Core Curriculum*).

While patients with suspected pulmonary tuberculosis are recovering from sedation or anesthesia after bronchoscopy, they must be placed in a separate AFB isolation room, not in a recovery room with other patients. Before the room may be used for another patient, sufficient time must be allowed to remove at least 99 percent of the airborne contaminants.

Similar precautions must be taken when other procedures are performed that may generate aerosols. Such procedures may include irrigation of tuberculosis abscesses, or laboratory procedures performed on liquids or tissues that may contain *M. tuberculosis.*

Diagnostic procedure: Mycobacteriology
Urine Collection

The first morning voided midstream specimen is preferred. Multiple specimens collected at daily intervals on at least three days are advised to demonstrate the presence of mycobacteria. It is preferable that the patient not be receiving broad-spectrum antibiotics at the time of collection because the antibiotics may inhibit growth of mycobacteria from urine.

Instruct the patient to collect at least 30-45 ml of urine. Submit by mail or Federal Express to the Missouri State Tuberculosis Laboratory in the containers supplied by the Laboratory.

Twenty-four-hour urine collections are unacceptable.

Diagnostic Procedure: Mycrobacteriology
Interpretation of Laboratory Reports

The information from the above documents has been adapted to the reporting methods of the Missouri State Tuberculosis Laboratory.

POLICY: Results from mycobacterial laboratories will be accurately interpreted according to appropriate protocols.

PURPOSE: To ensure accurate interpretation of laboratory reports from mycobacterial laboratories.

PROCEDURE: **Interpretation of results obtained from the Missouri State Tuberculosis Laboratory at Mount Vernon:**

SMEARS: A concentrated smear for acid-fast bacilli (AFB) will be examined on each specimen, except for gastric washes and urine, which is submitted to the Missouri State Tuberculosis Laboratory. The results of the smear will be received by telephone if positive, followed by a hard copy by mail, or by mail if negative. The reports will indicate the presence of AFB, **which may or may not be tubercle bacilli** were seen. The approximate quantity of organisms present will also be reported as:

None seen	Negative
10-30 per slide	1+; Rare
31-100 per slide	2+; Few
1-9/field	3+; Many
>9/field	4+; numerous

Observations made at 200X fluorochrome and calculated by this table equate with those made at 1000X oil immersion (Z-N or Kinyoun).

Persons with positive <u>sputum</u> smears are generally considered infectious, although persons with negative sputum smears and positive sputum cultures may also be infectious. AFB smears have a high specificity, but a rather low (60-70%) sensitivity. It is important to remember that a positive AFB sputum smear is only presumptive for pulmonary tuberculosis--a culture to positively identify the organism is imperative. However, a positive AFB sputum smear is <u>indicative</u> of pulmonary tuberculosis until proven otherwise. Immediate attention is required.

In body fluid other than sputum, a negative smear is not usually very meaningful. However, a positive smear is a very important clinical sign in body fluids other than sputum.
CULTURES: Culture of organisms is done regardless of AFB smear results. The culture must be done to positively identify the organism, and to determine drug susceptibilities. Negative smears may show growth of *M. tuberculosis* in culture media. **A positive culture for *M. tuberculosis* confirms the diagnosis of TB. However, the diagnosis may also be made on other criteria** (see Chapter 5 of *Core Curriculum*).

Both solid media and liquid medium (mycobacterial growth indicator TUB-MGIT) are used for all specimens at the Missouri State Tuberculosis Laboratory. The MGIT system can report a positive result in 10-14 days, but **negative results will take six weeks to be reported as "no growth".**

The initial isolate positive for *M. tuberculosis* is routinely tested for susceptibility to the four or five "first-line" anti-tuberculosis drugs, and susceptibility is repeated routinely when continued positive culture results are still obtained after two or three months of therapy. Additional susceptibilities are performed upon request or as necessary to determine whether drug resistance has evolved. Results of susceptibility tests are clearly reported as "Sensitive" or "Resistant." **All reports of drug resistance must be reported to the health care provider immediately for possible medication change. NEVER ALLOW THE ADDITION OF ONLY ONE ANTI-TUBERCULOSIS DRUG TO A REGIMEN FOR ANY PATIENT SHOWING ANY DRUG RESISTANCE, AS RESISTANCE TO MORE DRUGS WILL INEVITABLY DEVELOP.**

It is important to realize that specimens may show any of four (4) results:

Result	Interpretation
Smear -, culture -	**None to few bacilli present, nonviable**
Smear -, culture +	**Few bacilli present, but viable**
Smear +, culture -	**Bacilli present, but non-viable or not recovered**
Smear +, culture +	**Confirmed recovery of mycobacteria, identification required**

Laboratories other than the Missouri State Tuberculosis Laboratory:

SMEARS: Most laboratories will perform and report the results of a concentrated smear in the same way as the Missouri State Tuberculosis Laboratory does. Some laboratories will perform direct smears on specimens, which often does not provide an accurate result, as the specimen

may be too dilute to have adequate numbers of AFB on the slide, and are therefore easy to be undetected.

CULTURES: Many laboratories will perform only the smear and report the results, and send the rest of the specimen to another commercial laboratory or the Missouri State Tuberculosis Laboratory. Such routing may delay the resulting culture by a few days, or by many days if there is any problem with keeping the isolate alive in transit. Several commercial laboratories are located outside Missouri, and report only to the submitter and the state health department in which the laboratory is located, thereby delaying the report to the public health system in Missouri.

Most hospital laboratories are not equipped or staffed to perform drug susceptibilities. Many commercial laboratories must send the isolate to yet another laboratory for susceptibilities, thereby further delaying the results.

When it is known that a sputum specimen for AFB has been sent to a laboratory other than the State Tuberculosis Laboratory, it is helpful to notify the Section for Communicable Disease prevention, Disease Investigation Unit. The unit can then follow up to determine the results more rapidly.

For more information regarding MOTTS, refer to the ATS statement.

Diagnostic Procedure: Mycobacteriology
Distribution of Laboratory Reports

The Office of Surveillance receives telephone reports of all positive AFB smears from the Missouri State Tuberculosis Laboratory in Mount Vernon. Such reports are then immediately telephoned to the submitting agency or health care provider, with a hard copy to follow by mail. The district tuberculosis control nurse is also notified by electronic mail and immediately notifies the LPHA. Metropolitan areas that maintain register cards (TBC-4) and the central office will record all lab reports on the register cards (TBC-15) if the patient is a verified case.

Positive reports are forwarded by mail from the Office of Surveillance to the district health office and the LPHA. The district health nurse attaches it to the Case Register Card (TBC 15A), if the patient has been counted as a verified case. If the patient has not yet been reported as a case (on a CD-1 form), OoS will send an inter-office communication to the district health office requesting more information. The positive laboratory report is held in a "pending" file until the necessary information is received.

Negative reports are mailed to the District Health Office and LPHA.

Drug Sensitivity reports are mailed to the submitting agency or health care provider, and the Office of Surveillance. A copy of the report is forwarded by OoS to the district health office and the LPHA. The local health office must then ensure that the health care provider is aware of any resistance reported. Sensitivity reports are critical to the appropriate medical management of tuberculosis patients.

The Office of Surveillance also receives by mail some smear and culture reports from other laboratories, and handles these in the same way as reports from the Missouri State Tuberculosis Laboratory.